# BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

## IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.**

# Transgenic Animals

Edited by

F. GROSVELD

*Laboratory of Gene Structure and Expression, National Institute for Medical Research, London, UK*

and

G. KOLLIAS

*Molecular Genetics Laboratory, Hellenic Pasteur Institute, Athens, Greece*

ACADEMIC PRESS

*Harcourt Brace Jovanovich, Publishers*

London San Diego New York

B ston Sydney Tokyo Toronto

ACADEMIC PRESS LIMITED
24–28 Oval Road
London NW1 7DX

*United States Edition published by*
ACADEMIC PRESS INC.
San Diego, CA 92101



This book is printed on acid-free paper

A catalogue record for this book is available from the British Library

ISBN 0–12–304530–4

Typeset by Colset Private Limited, Singapore
Printed and bound in Great Britain by TJ Press Ltd, Padstow, Cornwall

# Conten

Contributors

Preface .....

1. **The R : G n m**

   A. E. SIP
   N. FAUS

   1. Intr
   2. Chr
   3. Reg
   4. Gen
   5. Con
      Ack
      Ref

2. **Dir cte R m**

   S. DOI, (

   1. Intr
   2. Ger
   3. Ext
   4. Arti
   5. Chr
   6. ES
   7. Str
   8. Scr
   9. Apl
   10. Cor
      Ref

# 9
# Oncogenesis and transgenic mice

D. KIOUSSIS

*Division of Molecular Immunology, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK*

Generation of *in vitro* cell lines using organ-directed oncogenesis ..... 197
Identifying cooperating oncogenes ..... 202
Circumventing difficulties in breeding and maintaining mice carrying harmful oncogenes ..... 204
Concluding remarks ..... 205
References ..... 206

## 1. Introduction

Oncogenes have been the focus of intense studies during the last 15 years. Initially, it was thought that the only biological activity of the viral oncogenes and their cellular homologues was to immortalize and transform cells which subsequently would develop into malignant or benign tumours. As research proceeded, however, it became evident that several of the cellular proto-oncogenes were implicated in cell differentiation (Westphal and Gruss, 1989), cell cycle (Coppola *et al.*, 1989) and embryonic development (Shackleford and Varmus, 1987; Wilkinson *et al.*, 1987; Mutter *et al.*, 1988). In recent years some of them were identified as transcription factors, such as *fos* and *jun* (Angel *et al.*, 1988). Studies on expression of such oncogenes in transfected cells has elucidated several aspects of the regulation of expression of these genes. However, their exact role in the processes referred to above remains unknown. Therefore, the deliberate introduction of such genes in the germ line of animals would provide more precise information.

Transgenic mice have been exceedingly useful in this respect. Generation of mice carrying oncogenes under the control of their own transcriptional elements has revealed tissue specificity, both in terms of their expression as well as their oncogenic action (Compere *et al.*, 1988; Cory and Adams, 1988; Hanahan, 1988,

TRANSGENIC ANIMALS
ISBN 0-12-304530-4

1989; Adams and Cory, 1991). It was however, the use of artificial hybrid genes which shed more light on their action and developed into useful tools in the study of cell differentiation and development. Such hybrid genes usually place the expression of the oncogene (activated, cellular, viral) under the control of an element which is either widely active, or of a restricted tissue specificity, i.e. a promoter with or without enhancer sequences. These constructs are introduced in the germ line of mice, and animals that carry these genes usually express the transoncogene in the tissue determined by the regulatory elements of the hybrid gene. This tissue often suffers a developmental disturbance and in most cases a tumour develops from the cells that express the oncogene.

Many such attempts have led to the expression of an activated cellular or viral oncogene in a variety of cell lineages or specific organs. For example, the simian virus 40 (SV40) large T antigen gene has been introduced in the germ line of mice under the control of many promoters, e.g insulin (Teitelman *et al.*, 1988), elastase (Ornitz *et al.*, 1987), α-crystallin (Mahon *et al.*, 1987), glucagon (Efrat *et al.*, 1988), metallothioneine (Messing *et al.*, 1985), or atrial natriuretic factor (Field, 1988) promoters among others. Transgenic animals were also generated which carried the *myc* gene under the control of: the immunoglobulin enhancer (Adams *et al.*, 1985; Suda *et al.*, 1987; Schmidt *et al.*, 1988), the mouse mammary tumour virus (MMTV) long terminal repeat (LTR) (Leder *et al.*, 1986), the H2 (Morello *et al.*, 1989) or *Thy-1* (Spanopoulou *et al.*, 1989) promoters; a *ras*-activated gene under the MMTV, whey acidic protein (WAP) (Andres *et al.*, 1987), elastase (Quaif *et al.*, 1987) or immunoglobulin transcription elements (Suda *et al.*, 1987; Harris *et al.*, 1988). More recently, other oncogenes have been studied in a similar fashion. Thus, expression of *neu* (Muller *et al.*, 1988; Bouchard *et al.*, 1989) and *fos* (Ruther *et al.*, 1987, 1988) has been targeted to specific tissues, almost invariably leading to tumours in the expressing organs.

In several of these animals the tumours were shown to be of monoclonal origin (Adams *et al.*, 1985; Leder *et al.*, 1986; Harris *et al.*, 1988; Teitelman *et al.*, 1988; Spanopoulou *et al.*, 1989). The transgene appears to be expressed in most cells of the organ where the tumours developed, yet not all cells proceed to malignancy. Thus, it became evident that additional events are needed in order to achieve the final transformed phenotype. These secondary events are most likely the expression of additional (onco) genes. More recently, some of the additional events were shown to be the inactivation of anti-oncogenes, such as the retinoblastoma gene (Bernards *et al.*, 1989; Hong *et al.*, 1989; Horowitz *et al.*, 1988). The concept of synergism (cooperativity) between oncogenes had already been demonstrated to be true in experiments with fibroblasts transfected with two oncogenes simultaneously (Land *et al.*, 1983). Transgenic animals expressing different oncogenes in the same tissue have proved instrumental in showing cooperativity between the oncogenes in question. Thus, transgenic animals harbouring the MMTV-*myc* hybrid gene were crossed with MMTV-*ras*

transgenic animals; the resulting double transgenics developed mammary tumours much faster than either of the single transgenics (Sinn *et al.*, 1987).

Excellent reviews on the results of transgenic experiments with oncogenes already exist in the literature and the reader is directed to some of those for additional information (Compere *et al.*, 1988; Cory and Adams, 1988; Hanahan, 1988, 1989; Adams and Cory, 1991).

In this chapter, three aspects of research with transgenic mice harbouring oncogenes will be considered. One aspect concerns the use of such mice to generate cell lines that have been difficult to obtain by other means. The second concerns the use of these mice to trap other cellular oncogenes which can cause cancer in cooperation with the transoncogene. The final aspect concerns the problem of breeding and maintaining mice which carry harmful oncogenes. These difficulties can be circumvented using carefully designed gene expression systems which lead to regulated expression of the oncogene product.

## 2. Generation of *in vitro* cell lines using organ-directed oncogenesis

Research on biological systems has been greatly enhanced by the availability of tissue-specific cell lines which can grow indefinitely *in vitro*. So far, such lines have been derived from tumours occurring spontaneously or induced by mutagens. Alternatively, retroviruses carrying oncogenes have been used to transform primary cell cultures. The advent of transgenic mice and the possibility to target the expression of genes in different organs using tissue-specific promoters made it feasible to induce tumours in mice in the tissue of choice. Thus, cell lines have been isolated from several of the transgenic mice which carry oncogenes under the control of different tissue-specific promoters (see references in Section 1). Below, the use of such animals to isolate cell lines from the thymus will be described.

Development of T cells takes place mainly in the thymus (Scollay *et al.*, 1984). Precursor cells arriving from fetal liver or bone marrow undergo a complex differentiation process resulting in mature functional T cells which are released in the periphery (Adkins *et al.*, 1987). During this development the surface phenotype of the thymocytes changes dramatically (Fowlkes and Pardoll, 1989). Thus, early cells express *Thy-1* and CD2 molecules on their membranes, but lack some of the mature markers found in circulating T lymphocytes, such as CD4 and CD8. Therefore, they are known as double-negative and this population (approximately 2–4% of thymocytes) appears to contain most of the precursor cells that can repopulate a depleted thymus. Later in development these cells acquire CD4 and CD8 simultaneously on their surface and start expressing low levels of the T cell receptor complex (TcR$\alpha\beta$ and CD3$\gamma\delta\varepsilon\zeta$n

chains). This population, by far the most abundant in the thymus (80–90%), are known as double positives and represent an intermediate step between precursors and mature T cells. Many of these cells are destined to die within the thymus. A minority (approximately 10–20%) of them mature into single-positive $CD4^+CD8^-$ or $CD4^-CD8^+$ mature T cells with high levels of functional T cell receptor. Another smaller subset of T cells appears to have a different receptor on the cell surface, comprised of $\gamma$ and $\delta$ chains.

Mature T cells are capable of recognizing foreign antigens only in the context of their own MHC products (class I or class II), a phenomenon known as MHC restriction (Alison and Lanier, 1987). MHC restriction is the result of two major mechanisms: positive selection, during which functional cells are allowed to mature, and negative selection, during which harmful cells are eliminated or rendered functionally disabled (anergy) (Ramsdell *et al.*, 1989).

During the described thymic maturation process, it has become clear that the thymic microenvironment plays an important role. The stroma of the thymus is composed of a heterogeneous population of epithelial cells of unknown origin which are found throughout the cortex and the medulla of the thymus. It is widely thought that these stromal cells are not just a supporting structural element, but actively participate in the development of T cells, playing a central role in positive and negative selection. The heterogeneity of the stromal cells is manifested both in morphology and in expression of specific markers. Thus, there are monoclonal antibodies which can distinguish between cortical and medullary epithelial cells (Haynes, 1984; Lampert and Ritter, 1988).

The study of interaction of thymic stroma with the developing thymocytes in whole animals has been hampered by the heterogeneity of the microenvironment cells as well as by the contemporaneous existence of lymphocytes at different stages of development. Such *in vivo* studies, however, have elucidated the precursor–progeny relationship of some of the thymocyte subpopulations (Scollay *et al.*, 1984; Bluestone *et al.*, 1987), but the factors and conditions determining the transition from precursor to product are still unknown. This is probably accomplished by cell surface interactions between the developing thymocytes and stromal cells, which either trigger responses by themselves or result in the production of cytokines that determine the subsequent events in development.

Experimental systems which can reproduce *in vitro* some of the steps occurring in the thymocyte development, therefore, would be of great assistance in understanding the underlying mechanisms. Immortalized cells from the thymus, including both stromal cells as well as precursor or immature thymocytes, can be derived by immortalization/transformation after introduction into their genome of an oncogene. This theoretically could be accomplished by transfection or retroviral infection of primary cultures of the organs. However, such an approach becomes very difficult when the cells of interest represent a small

min rity or when they have a limited life span in primary cultures. Transgenic mice harbouring an oncogene under the control of tissue-specific transcripti nal elements provide an alternative pathway to the development of such cell lines. This has proved a successful approach in the past and cell lines have been isolated from mice carrying trans-oncogenes under the control of tissue-specific promoters (see references above)

This is illustrated by the experimental system in which transgenic mice were generated which carry the *c-myc* gene under the control of the *Thy-1* gene (Spanopoulou *et al.*, 1989). Such transgenic mice express the *myc* gene in high levels in the thymus and develop thymic tumours between 6 and 16 weeks of age (depending on the levels of *myc* expression). Immunohistochemical analysis of these tumours showed that both stromal and lymphoid components had expanded in the tumour. Fluorescence activated cell sorter (FACS) analysis of the lymphoid component revealed that most of the cells were $CD4^+8^+$ or $CD4^+8^-$ (Fig. 1(c)). The transformed phenotype of the cells in such tumours was manifested by the ability of homogenates of such thymuses to cause tumours in histocompatible mice and by their ability to establish in culture both adherent and suspension cell lines.

The adherent cell lines morphologically appeared as 'cobblestone pavement', which is typical of epithelial cells, and stained with antibodies which stain cortical epithelial cells *in vivo* (Lampert and Ritter, 1988). The suspension cell lines exhibit markers of the lymphoid lineage. Thus, the majority of the lines are double-positive ($CD4^+CD8^+$) and express on their surface high levels of heat-stable antigen (HSA, Jlld) which is a marker for immature thymocytes. They also express low levels of T cell receptor complex, in accordance with their classification as precursor-type thymocytes. Southern blot analysis of DNA from the cell lines, as well as from thymic tumours using a TcR$\beta$ constant-region probe, revealed that they are of mono- or oligoclonal origin, testifying further to the transformed phenotype of these cells. In some tumours which were composed mostly of $CD4^+CD8^+$ and $CD4^+8^-$ cells it was found that the same $\beta$ chain rearrangement was present in the DNA of these two subpopulations. This is strong evidence that the double-positive cells isolated from these tumours have the capability to mature into single-positive cells, given the right environment.

Interestingly, the adherent lines, apart from their epithelial phenotype, exhibited an additional property which makes them a good candidate for an *in vitro* differentiation system. When co-cultured with established double-positive cell lines from a *Thy–myc* tumour, they would rosette the lymphocytes in a strong and specific manner. In the same culture lymphocytes would ignore colonies of fibroblastic morphology (Fig. 2). This specific interaction between the epithelial cell lines and the lymphoid precursor lines can be exploited in differentiation experiments involving the appropriate MHC haplotype on the epithelial cells and the appropriate T cell receptor in the precursor thymocytes.

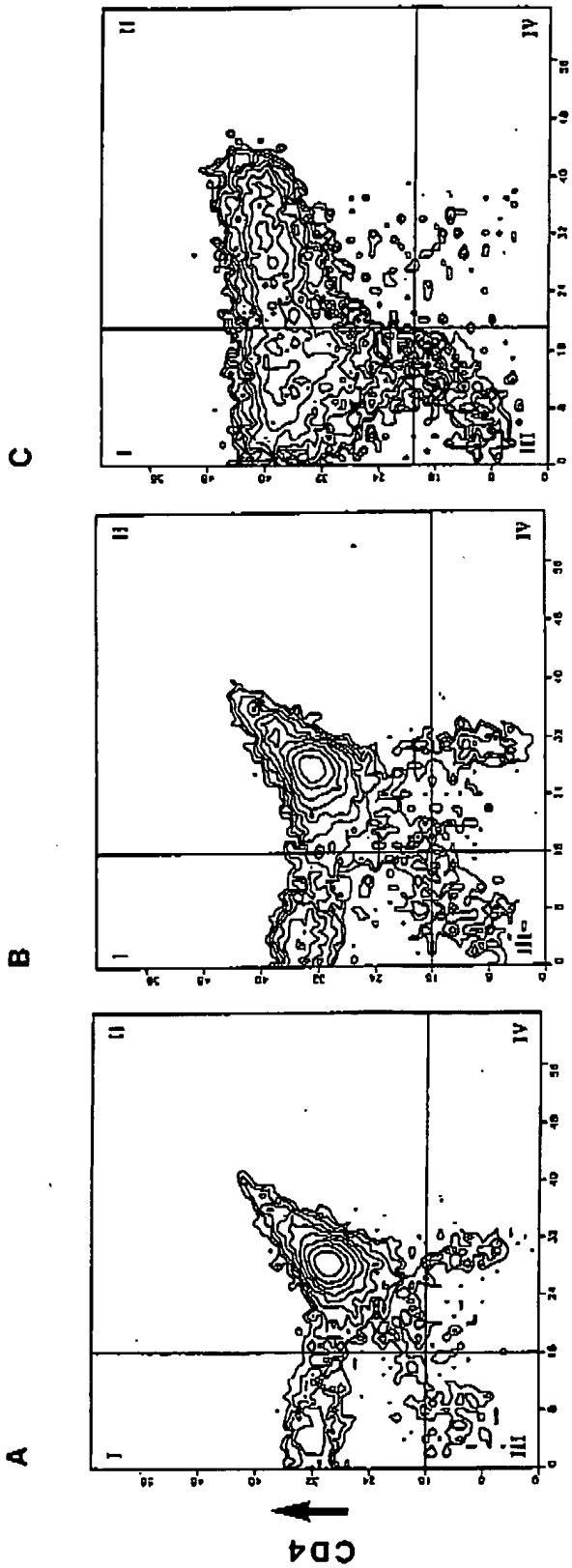


*Fig. 1.* Contour plots of CD4 and CD8 expression on thymocytes from (A) normal mouse, (B) H2-tsA58 and (C) *Thy-myc* transgenic mice.

*Fig. 2.* Specific in
lines (right). In th

In an exten
oncogenesis, a
thermolabile n
genic mice (Jat
most potent in
which carry th
see Compere *e*
all these cases,
where the expr
due to tumour
rise to protein
Since the body
molecules mad
dissected, how
i.e. 33°C, then
ensure the expr
use of the MH
*et al.*, 1986; D

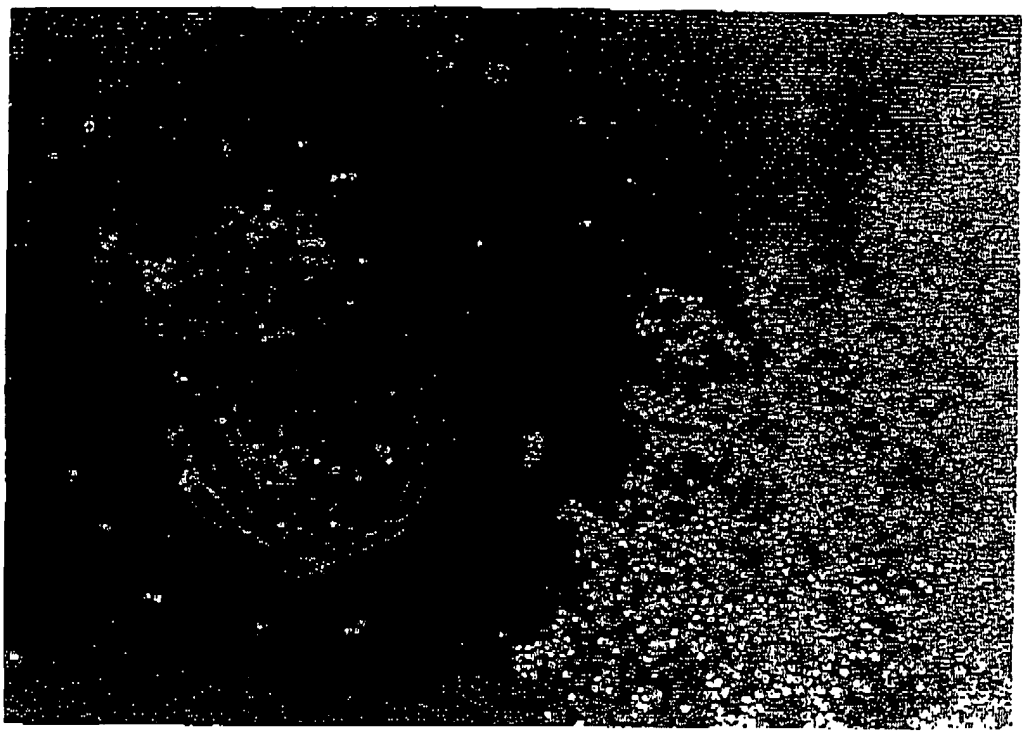

*Fig. 2.* Specific interaction of double positive ($CD4^+ 8^+$) thymocytes with thymic epithelial cell lines (right). In the same co-culture the lymphocytes do not adhere to the fibroblastic colony (left).

In an extension of this principle of obtaining cell lines through targeted oncogenesis, another construct was devised which ensured the expression of a thermolabile mutant of the SV40 T antigen in a wide variety of tissues in transgenic mice (Jat *et al.*, 1991). The SV40 large T antigen (Tag) gene is one of the most potent immortalizing genes. Several transgenic mice have been generated which carry the wild type of the gene under a variety of promoters (for reviews see Compere *et al.*, 1988; Cory and Adams, 1988; Hanahan, 1989). In almost all these cases, tumours developed in the transgenic mice, usually in the tissues where the expression of the Tag was targeted. In order to avoid the lethal effects due to tumours caused by the Tag, a mutant gene *TsA58* was used which gives rise to protein which is unstable at 39°C and stable at 33°C (Tegtmeyer, 1975). Since the body temperature of the mouse is 37.5°C, the majority of the Tag molecules made in the transgenic mouse would be degraded. If the tissues were dissected, however, and placed in culture conditions which stabilize the protein, i.e. 33°C, then the Tag would be allowed to immortalize the cells. In order to ensure the expression of the transgene in as wide a variety of tissues as possible, use of the MHC class I $H2^k$ promoter was made (Weiss *et al.*, 1983; Kimura *et al.*, 1986; David-Watinne *et al.*, 1990). This promoter has the additi nal

advantage that it can be induced by γ-interferon, thus offering one more level of control (Wallach *et al.*, 1982; Israel *et al.*, 1986; David-Watinne *et al.*, 1990). Indeed, transgenic mice harbouring this hybrid H2-tsA58 construct expressed Tag mRNA in a wide variety of tissues, with thymus and liver ranking amongst the highest expressing tissues (Jat *et al.*, 1991). Skin fibroblasts from these mice were placed at 33°C in the presence of low levels of γ-interferon (1 U $ml^{-1}$), and yielded immortalized cell lines, which were conditional in their growth. Thus, they would grow continuously without undergoing crisis in the permissive conditions, but would stop dividing when placed at 39°C or in cultures devoid of γ-interferon.

Whereas the rest of the tissues were normal, these mice invariably suffered from thymic hyperplasia. The likely absence of transformation from these thymuses was shown by the following observations: (1) both lobes of the thymus were equally enlarged; (2) unlike the *Thy-myc* thymic tumours, homogenates of these thymuses were unable to cause tumours in histocompatible animals, even when $10^7$ cells were injected subcutaneously or intraperitoneally; (3) another difference from the *Thy–myc* tumours was the fact that the enlarged thymuses had polyclonal populations of thymocytes as judged by Southern blot analysis of their β chain gene loci; (4) furthermore, fluocytometry using antibodies against lymphocyte surface markers showed normal distribution of the major thymus subpopulations (Fig. 1(a) and (b)).

All this evidence taken together indicates that the lymphocytic expansion in these enlarged thymuses is probably of a hyperplastic nature rather than neoplastic. The mono- or polyclonality of the epithelial cells which also expand in these thymuses is not easy to determine. However, as mentioned above, homogenates of the enlarged thymuses cannot cause transplantable tumours; therefore, the epithelial cells included in the injection are unlikely to be transformed. The homogenates of these thymuses were put in culture at 33°C in the presence of interferon and they yielded cultures of cells which are keratin-positive and exhibit the typical morphology of thymic epithelial cells. These cultures are also conditional in their growth, in that they stop dividing if they are placed in 39°C. Given the wide tissue distribution of the expression of this transgene in these mice, it is very interesting to see which other tissues can yield conditional cell lines and which cannot.

## 3. Identifying cooperating oncogenes

Expression of a transgene in a tissue usually does not guarantee the generation of tumours from all cells in this tissue. Thus, Eμ–*myc* mice develop certain pre-B cell tumours, but not other B cell malignancies (Suda *et al.*, 1987; Harris *et al.*, 1988). Similarly, *Thy–myc* mice devel p thymic tumours f predominantly

double-positive ($CD4^+CD8^+$) phenotype (Spanopoulou *et al.*, 1989). It is possible that in both f these cases the *myc* gene causes an increase f these populations of cells and thus increasing the probability that secondary events which lead to the final transformation.

In the above cases the secondary event is of an unknown nature. However, sometimes cooperation between oncogenes has been directly shown by crossing different transgenic mice; thus, when mice which express the *myc* transgene in the mammary gland are crossed with transgenic mice expressing the *ras* gene in the same tissue, they develop mammary tumours faster than either parental strain (Teitelman *et al.*, 1988). Similarly, transgenic mice expressing both *myc* and *ras* in their B cells develop pre-B lymphomas at a faster rate than mice expressing either of the genes alone in this compartment (Cory and Adams, 1988). A similar situation occurs when mice carrying the *Thy–myc* hybrid gene are crossed to transgenic mice with the activated H-*ras* gene under the control of the hCD2 gene. The double-transgenic offspring develop thymic tumours much earlier than either of the single transgenic parents (D. Greenberg and D. Kioussis, unpublished observations). Interestingly, the lymphomas developed in this case were monoclonal in origin, indicating that additional events have to take place in order to transform the cells.

In order to be able to identify such additional genetic events, Anton Berns and his colleagues devised a protocol which uses transgenic animals carrying an oncogene and infecting them with a slow-transforming retrovirus devoid of oncogenes (Berns *et al.*, 1989). In this protocol the resident oncogene in the transgenic mice was *pim-1* under the control of its own promoter, the Eμ enhancer and the Moloney murine leukaemia virus (Mo-MuLV) LTR. Such mice express high levels of the transgene in their haemopoietic tissues. However, these mice show no abnormalities in their haemopoietic or lymphoid organs in the early stages (van Lohuizen *et al.*, 1988). Only after a latency period, which can last up to 8 months, do 10% of the transgenic mice develop T cell lymphomas. Thus, the tumour incidence in the Eμ–*pim-1* transgenic mice is very low. Such mice are ideal to use for identification of other genes whose expression or extinction will synergize with *pim* expression and lead to tumour formation. In order to tag such genes, Berns and his colleagues infected newborn normal or *pim* transgenic mice with Mo-MuLV. All the Eμ–*pim-1* transgenic animals developed lymphomas much earlier than their non-transgenic littermates. These lymphomas were oligoclonal and had T cell characteristics. The authors concluded that the Eμ–*pim-1* presence in the genome of these mice rendered them highly susceptible to lymphoma induction by Mo-MuLV. Further investigation of these tumours established that the majority of the proviruses were integrated near the c-*myc* gene, causing transcriptional activation of this oncogene. The activation of the *myc* gene in this case is (one of) the necessary event(s) that leads t the tumour formation. Thus, the synergism between the *pim-1* and *myc* genes

is documented and verified by this system. To further document the synergy between these two oncogenes, the authors proceeded to infect E$\mu$–*myc* transgenic mice with Mo-MuLV. The non-infected mice develop spontaneous pre-B cell lymphomas and infection by Mo-MuLV accelerated the incidence of such lymphomas. When analysed, it was found that, in a significant fraction of the lymphomas, the *pim-1* gene was activated by the proviral insertion. Such experiments indicate that the Mo-MuLV infection of transgenic mice bearing oncogenes can be exploited to identify other oncogenes which cooperate with the resident trans-oncogene in tumorigenesis. It will be highly interesting to see whether inactivation of anti-oncogenes by retroviral insertion can be detected by this protocol.

## 4. Circumventing difficulties in breeding and maintaining mice carrying harmful oncogenes

In the examples quoted above, the animals expressing a trans-oncogene in their tissues almost invariably develop tumours. The latency of such tumours sometimes is short and the tumour can cause physiological disturbances which make maintenance and breeding of these animals problematic. Therefore, it would be of advantage to introduce a gene in the germ line which remains inactive during the life of the animal, but can be activated at will. This could be achieved by the use of temperature-sensitive variants of oncogenes or by placing the oncogene under the control of an element which can be transactivated. In the first case, the inactive transgene (the thermolabile protein) in the animal can be activated by placing the tissues in permissive culture conditions. Such an experimental animal was described above and harbours in its genome a temperature-sensitive SV40 T-antigen gene, *TsA58* (Jat *et al.*, 1991).

The second type of transgenic mouse was attempted in several laboratories using mammalian virus transactivating systems. Thus, transgenic mice were generated which carry the reporter gene chloramphenicol acetyltransferase (CAT) under the control of the human immunodifficiency virus (HIV) LTR, or the HIV *tat* gene under a regulatory element from the αA crystallin gene. When these two strains were crossed, transactivation of the reporter gene by the *tat* product caused expression of CAT in the eyes of double-transgenic mice (Khillan *et al.*, 1988). In another set of experiments, the promoter of an immediate early gene of herpes simplex virus (HSV-1) was transactivated by the VP16 gene product in double-transgenic mice (Byrne and Ruddle, 1989). However, the regulatory elements of mammalian viruses are subject to effects from the transcriptional machinery of the mouse cells. In order to avoid problems of unwanted expression, Leder and his colleagues decided to use regulat ry elements from distantly related species (Ornitz *et al.*, 1990). Thus, firstly, a

construct is made which places the onc gene of interest under the control of regulatory elements that only respond to the yeast transcriptional activat r GAL4 (Ptashne, 1986; Kakidani and Ptashne, 1988; Byrne and Ruddle, 1989). The promoter of such a construct is engineered in such a way that the transgene remains silent in mammalian cells or transgenic mice harbouring it. This construct constitutes the target gene and it can only be expressed in the presence of the transcriptional activator GAL4.

In a second step in this protocol, another transgenic mouse is generated (the transactivator animal). This mouse harbours the yeast *GAL4* gene under the control of specific viral or mammalian transcriptional regulatory elements. The choice of the promoter and the regulatory elements can be designed so that expression of the *GAL4* gene is restricted to a few tissues or at certain times during development. On the other hand, it can be designed to be expressed in a wide variety of tissues. If one mates the two animals described above (the target and transactivator mice), their offspring containing the two transgenes will express the oncogene only in those tissues expressing the *GAL4* gene.

The above protocol was tested using as the target animal a transgenic mouse carrying the *Int-2* gene under the control of GAL4 responsive transcriptional elements. *Int-2* has been implicated in mammary tumorigenesis (Dickson *et al.*, 1984; Ali *et al.*, 1989), and recently in mouse development (Jakobovitz *et al.*, 1986; Wilkinson *et al.*, 1988). When this gene is over-expressed in breast or prostate tissues of transgenic mice it causes an enlargement of the mammary or prostate gland, characterized by hyperplasia of their epithelium (Muller *et al.*, 1990). Mice which carry the GAL4-inducible *Int-2* gene are normal and they do not exhibit any expression of the transgene in their tissues.

The transactivator mouse carries the mouse mammary tumour virus LTR fused to a mutant version of *GAL4* (*GAL4*/236). The presence of the LTR in this construct ensures the expression of fairly high levels of GAL4 in several tissues of the transgenic mouse (Leder *et al.*, 1986).

Offspring of a cross between these two transgenic mice carrying both transgenes express the target gene (*Int-2*) in all tissues that express GAL4. This causes hyperplasia in salivary glands, breast tissue, epididymis and prostate. Such double-transgenic mice have problems reproducing or rearing their offspring, testifying to the usefulness of this binary system.

## 5. Concluding remarks

Transgenic animals have been proved to be an excellent tool in studying gene regulation and function. Particularly useful applications were employed in studies using oncogenes. In this chapter we describe how such transgenic animal models can be used as tools to answer questions r facilitate procedures which

have been problematic in the past, such as isolation f imm rtalized cell lines. Regulated expression of oncogenes allows the development of normal tissues in mice until the chosen time. Excision of tissues and placement under permissive conditions yields immortalized lines from *Hs-TsA58* transgenic mice. Alternatively, mating of carefully designed target-transactivating mice results in tumours only in the tissues of interest.

Finally, transgenic animals carrying oncogenes can be used to track down additional oncogenes that cooperate with the transgene or to identify loss of function mutants in anti-oncogene loci.

## References


Adams, J. M. and Cory, S. (1991). Transgenic models for haemopoietic malignancies. *Biochim. Biophys. Acta* **1072**, 9–31.

Adams, J. M., Harris, A. W., Pinkert, C. A., Corcoran, L. M., Alexander, W. S., Cory, S., Palmiter, R. D. and Brinster, R. L. (1985). The c-myc oncogene driven by immunoglobulin enhancers induces lymphoid malignancy in transgenic mice. *Nature* **318**, 533–538.

Adkins, B., Mueller, C., Okada, C. Y., Reichert, R. A., Weissman, I. L. and Spangrude, G. J. (1987). Early events in T-cell maturation. *An. Rev. Immunol.* **5**, 325–365.

Ali, I. U., Merlo, G., Callahan, R. and Lidereau, R. (1989). The amplification unit on chromosome 11q13 in aggressive primary human breast tumors entails the bcl-1, int-2 and hst loci. *Oncogene* **4**, 88–92.

Alison, J. P. and Lanier, L. L. (1987). Structure, function and serology of the T-cell antigen receptor complex. *An. Rev. Immunol.* **5**, 503–540.

Andres, A. C., Schonenberger, C. A., Groner, B., Henninghausen, L., LeMeur, M. and Gerlinger, P. (1987). Ha-ras oncogene expression directed by a milk protein gene promoter: tissue specificity, hormonal regulation, and tumor induction in transgenic mice. *Proc. Natl Acad. Sci. USA* **84**, 1299–1303.

Angel, P., Allegretto, E., Okino, S. T., Hattori, K., Boyle, W. J., Hunter, T. and Karin, M. (1988). Oncogene jun encodes a sequence-specific trans-activator similar to AP-1. *Nature* **332**, 166–171.

Bernards, R., Schackleford, G. M., Geiber, M. R., Horowitz, J. M., Friend, S. H., Schartl, M., Bogenmann, E., Rapaport, J. M., McGee, T., Dryja, T. P. and Weinberg, R. A. (1989). Structure and expression of the murine retinoblastoma gene and characterization of its encoded protein. *Proc. Natl Acad. Sci. USA* **86**, 6474–6478.

Berns, A., Breuer, M., Verbeek, S. and van Lohuizen, M. (1989). Transgenic mice as a means to study synergism between oncogenes. *Int. J. Cancer* Supp. **4**, 22–25.

Blackman, M. A., Gerhard-Burgert, H., Woodland, D. L., Palmer, E., Kappler, J. W. and Marrack, P. (1990). A role for clonal inactivation in T cell tolerance to Mls-1a. *Nature* **345**, 540–542.

Blackman, M. A., Kappler, J. W. and Marrack, P. (1988). T-cell specificity and repertoire *Immunol. Rev.* **101**, 5–19.

Bluestone, T.
tion of n
326, 82-
Bouchard, L
appearan
oncogen
Byrne, G. W.
to transg
Compere, S.
*Biochim.*
Coppola, J. A
from the
blocked
Cory, S. and
6, 25–48
David-Watinr
of MHC
Dickson, C.,
mammar
tion regi
Efrat, S., Tei
gene reg
alpha cel
Field, L. J. (1
and card
Fowlkes, B. J.
ment. *A*
Hanahan, D.
*Genet.* 2
Hanahan, D.
1265–12
Harris, A. W.
Vaux, D.
S. (1988)
*Microbiol*
Haynes, B. F.
Hong, F. D.,
Lee, W.
*Sci. USA*
Horowitz, J. M
E. (1988)
*Harbor S*
Israel, A., Kir
response
mouse H
Jakobovitz, A

Bluestone, T. A., Pardoll, D. M., Sharrow, S. O., and Fowlkes, B. J. (1987). Characterization of murine thymocytes with CD3-associated T-cell receptor structures. *Nature* **326**, 82–84.

Bouchard, L., Lammarre, L., Tremblay, P. J. and Jolicoeur, P. (1989). Stochastic appearance of mammary tumors in transgenic mice carrying the MTV/c-neu oncogene. *Cell* **57**, 931–936.

Byrne, G. W. and Ruddle, F. H. (1989). Multiplex gene regulation: a two-tiered approach to transgene regulation in transgenic mice. *Proc. Natl Acad. Sci. USA* **86**, 5473–5477.

Compere, S. J., Baldacci, P. and Jaenisch, R. (1988). Oncogenes in transgenic mice. *Biochim. Biophys. Acta* **948**, 129–149.

Coppola, J. A., Parker, J. M., Schuler, G. D. and Cole, M. (1989). Continued withdrawal from the cell cycle and regulation of cellular genes in mouse erythroleukemia cells blocked in differentiation by the c-myc oncogene. *Mol. Cell. Biol.* **9**, 1714–1720.

Cory, S. and Adams, J. M. (1988). Transgenic mice and oncogenesis. *An. Rev. Immunol.* **6**, 25–48.

David-Watinne, B., Israel, A. and Kourilsky, P. (1990). The regulation and expression of MHC class I genes. *Immunol. Today* **11**, 286–292.

Dickson, C., Smith, R., Brookes, S. and Peters, G. (1984). Tumorigenesis by mouse mammary tumor virus: proviral activation of a cellular gene in the common integration region *int-2*. *Cell* **37**, 529–536.

Efrat, S., Teitelman, G., Anwar, M., Ruggiero, D. and Hanahan, D. (1988). Glucagon gene regulatory region directs oncoprotein expression to neurons and pancreatic alpha cells. *Neuron* **1**, 605–613.

Field, L. J. (1988). Atrial natriuretic factor-SV40 T antigen transgenes produce tumors and cardiac arrhythmias in mice. *Science* **239**, 1029–1033.

Fowlkes, B. J. and Pardoll, D. M. (1989). Molecular and cellular events of T cell development. *Adv. Immunol.* **44**, 207–264.

Hanahan, D. (1988). Dissecting multistep tumorigenesis in transgenic mice. *An. Rev. Genet.* **22**, 479–519.

Hanahan, D. (1989). Transgenic mice as probes into complex systems. *Science* **246**, 1265–1275.

Harris, A. W., Langdon, W. Y., Alexander, W. S., Hariharan, I. K., Rosenbaum, H., Vaux, D., Webb, E., Bernard, O., Crawford, M., Abud, H., Adams, J. M. and Cory, S. (1988). Transgenic mouse models for hematopoietic tumorigenesis. *Curr. Top. Microbiol. Immunol.* **141**, 82–93.

Haynes, B. F. (1984). The human thymic microenvironment. *Adv. Immunol.* **36**, 87–142.

Hong, F. D., Huang, H. J., To, H., Young, L. J., Oro, A., Bookstein, R., Lee, E. Y. and Lee, W. H. (1989). Structure of the human retinoblastoma gene. *Proc. Natl Acad. Sci. USA* **86**, 5502–5506.

Horowitz, J. M., Friend, S. H., Weinberg, R. A., Whyte, P., Buchkovich, K. and Harlow, E. (1988). Anti-oncogenes and the negative regulation of cell growth. *Cold Spring Harbor Symp. Quant. Biol.* **53** (part 2), 843–847.

Israel, A., Kimura, A., Fournier, A., Fellous, M. and Kourilsky, P. (1986). Interferon response sequence potentiates activity of an enhancer in the promoter region of a mouse H-2 gene. *Nature* **322**, 743–746.

Jakobovitz, A., Shackleford, G. M., Varmus, H. E. and Martin, G. R. (1986). Two

proto-oncogenes implicated in mammary carcinogenesis, int-1 and int-2, are independently regulated during mouse development. *Proc. Natl Acad. Sci. USA* **83**, 7806–7810.

Jat, P. S., Noble, M. D., Ataliotis, P., Tanaka, Y., Yannoutsos, N., Larsen L. and Kioussis, D. (1991). Direct derivation of conditionally immortal cell lines from an H-2K$^b$tsA58 transgenic mouse. *Proc. Natl Acad. Sci. USA* **88**, 5096–5100.

Kakidani, H. and Ptashne, M. (1988). GAL4 activates gene expression in mammalian cells. *Cell* **52**, 161–167.

Khillan, J. S., Deen, K. C., Yu, S., Sweet, R. W., Rosenberg, M. and Westphal, H. (1988). Gene transactivation mediated by the TAT gene of human immunodeficiency virus in transgenic mice. *Nucl. Acids Res.* **16**, 1423–1430.

Kimura, A., Israel, A., LeBail, O. and Kourilsky, P. (1986). Detailed analysis of the mouse H-2Kb promoter: enhancer-like sequences and their role in the regulation of class I gene expression. *Cell* **44**, 261–272.

Lampert, I. A. and Ritter, M. A. (1988). The origin of the diverse epithelial cells of the thymus; is there a common stem cell? *Thymus Update* **1**, 5–25.

Land. H., Parada, L. F. and Weinberg, R. A. (1983). Tumorigenic conversion of primary embryo fibroblasts requires at least two co-operating oncogenes. *Nature* **304**, 596–602.

Leder, A., Pattengale, P. K., Kuo, A., Stewart, T. A. and Leder, P. (1986). Consequences of widespread deregulation of the c-myc gene in transgenic mice: multiple neoplasms and normal development. *Cell* **45**, 485–495.

Mahon, K. A., Chepelinsky, A. B., Khillan, J. S., Overbeek, P. A., Piatigorsky, J. and Westphal, H. (1987). Oncogenesis of the lens in transgenic mice. *Science* **235**, 1622–1628.

Messing, A., Chen, H. Y., Palmiter, R. D. and Brinster, R. L. (1985). Peripheral neuropathies, hepatocellular carcinomas and islet cell adenomas in transgenic mice. *Nature* **316**, 461–463.

Morello, D., Lavenu, A., Bandeira, A., Portnoi, D., Gaillard, J. and Babinet, C. (1989). Lymphoproliferative syndrome associated with c-myc expression driven by a class I gene promoter in transgenic mice. *Oncogene Res.* **4**, 111–125.

Muller, W. J., Lee, F. S., Dickson, C., Peters, G., Pattengale, P. and Leder, P. (1990). The int-2 gene product acts as an epithelial growth factor in transgenic mice. *EMBO J.* **9**, 907–913.

Muller, W. J., Sinn, E., Pattengale, P. K., Wallace, R. and Leder, P. (1988). Single-step induction of mammary adenocarcinoma in transgenic mice bearing the activated c-neu oncogene. *Cell* **54**, 105–115.

Mutter, G. L., Grills, G. S. and Wolgemuth, D. J. (1988). Evidence for the involvement of the proto-oncogene c-mos in mammalian meiotic maturation and possibly very early embryogenesis. *EMBO J.* **7**, 683–689.

Ornitz, D. M., Hammer, R. E., Messing, A., Palmiter, R. D. and Brinster, R. L. (1987). Pancreatic neoplasia induced by SV40 T-antigen expression in acinar cells of transgenic mice. *Science* **238**, 188–193.

Ornitz, D. M., Moreadith, R. W. and Leder, P. (1990). Binary system for regulating transgene expression in mice: targeting int-2 gene expression with yeast GAL4/UAS control elements. *Proc. Natl Acad. Sci. USA* **88**, 698–702.

Ptashne, M. (19
**322**, 697–7
Quaife, C. J., Pi
Pancreatic
**48**, 1023–1
Ramsdell, F., L
self toleran
Ruther, U., G
Deregulate
mice. *Natu*
Ruther, U., Mu
(1988). c-fo
**53**, 847–85
Schmidt, E. V.,
the human
lymphoma
Scollay, R., Bart
thymus: ch
during dev
79–103.
Shackleford, G.
is restricted
embryos. C
Sinn, E., Mulle
Coexpressi
synergistic
Spanopoulou, E
Grosveld, I
tumours in
Suda, Y., Aizaw
Ikawa, Y. (1
lung adeno
gene a vari
Tegtmeyer, P. (
*J. Virol.* 15
Teitelman, G.,
neoplastic p
van Lohuizen, M
T. and Ber
mice: coope
*Cell* **56**, 67
Wallach, D., Fe
the synthes
833–836.
Weiss, E. H., M
R. A. (1983

Ptashne, M. (1986). Gene regulation by proteins acting nearby and at a distance. *Nature* **322**, 697–701.

Quaife, C. J., Pinkert, C. A., Ornitz, D. M., Palmiter, R. D. and Brinster, R. L. (1987). Pancreatic neoplasia induced by ras expression in acinar cells of transgenic mice. *Cell* **48**, 1023–1034.

Ramsdell, F., Lantz, T. and Fowlkes, B. J. (1989). A nondeletional mechanism of thymic self tolerance. *Science* **246**, 1038–1041.

Ruther, U., Garber, C., Komitowski, D., Muller, R. and Wagner, E. F. (1987). Deregulated c-fos expression interferes with normal bone development in transgenic mice. *Nature* **325**, 412–416.

Ruther, U., Muller, W., Sumida, T., Tokuhisa, T., Rajewsky, K. and Wagner, E. F. (1988). c-fos expression interferes with thymus development in transgenic mice. *Cell* **53**, 847–856.

Schmidt, E. V., Pattengale, P. K., Weir, L. and Leder, P. (1988). Transgenic mice bearing the human c-myc gene activated by an immunoglobulin enhancer; a pre-B-cell lymphoma model. *Proc. Natl Acad. Sci. USA* **85**, 6047–6051.

Scollay, R., Bartlett, P. and Shortman, K. (1984). T-cell development in the adult murine thymus: changes in the expression of the surface antigens Ly2, L314, and B242 during development from early precursor cells to emigrants. *Immunol. Rev.* **82**, 79–103.

Shackleford, G. M. and Varmus, H. E. (1987). Expression of the proto-oncogene int-1 is restricted to postmeiotic male germ cells and the neural tube of mid-gestational embryos. *Cell* **50**, 89–95.

Sinn, E., Muller, W., Pattengale, P., Tepler, I., Wallace, R. and Leder, P. (1987). Coexpression of MMTV/v-Ha-ras and MMTV/c-myc genes in transgenic mice: synergistic action of oncogenes in vivo. *Cell*, **49**, 465–475.

Spanopoulou, E., Early, A., Elliott, J., Crispe, N., Ladyman, H., Ritter, M., Watt, S., Grosveld, F. and Kioussis, D. (1989). Complex lymphoid and epithelial thymic tumours in Thy1-myc transgenic mice. *Nature* **342**, 185–189.

Suda, Y., Aizawa, S., Hirai, S., Inoue, T., Furuta, Y., Suzuki, M., Hirohashi, S. and Ikawa, Y. (1987). Driven by the same Ig enhancer and SV40 T promoter ras induced lung adenomatous tumors, myc induced pre-B cell lymphomas and SV40 large T gene a variety of tumors in transgenic mice. *EMBO J.* **6**, 4055–4065.

Tegtmeyer, P. (1975). Function of simian virus 40 gene A in transforming infection. *J. Virol.* **15**, 613–618.

Teitelman, G., Alpert, S. and Hanahan, D. (1988). Proliferation, senescence, and neoplastic progression of beta cells in hyperplasic pancreatic islets. *Cell* **52**, 97–105.

van Lohuizen, M., Verbeek, S., Krimpenfort, P., Domen, J., Saris, C., Radaszkiewicz, T. and Berns, A. (1988). Predisposition to lymphomagenesis in pim-1 transgenic mice: cooperation with c-myc and N-myc in murine leukemia virus-induced tumors. *Cell* **56**, 673–682.

Wallach, D., Fellous, M. and Revel, M. (1982). Preferential effect of γ-interferon on the synthesis of HLA antigens and their mRNAs in human cells. *Nature* **299**, 833–836.

Weiss, E. H., Mellor, A., Golden, L., Fahrner, K., Simpson, E., Hurst, J. and Flavell, R. A. (1983). The structure of a mutant H-2 gene suggests that the generation of

polymorphism in H-2 genes may occur by gene conversion-like events. *Nature* **301**, 671–674.

Westphal, H. and Gruss, P. (1989). Molecular genetics of development studied in the transgenic mouse. *An. Rev. Cell. Biol.* **5**, 181–196.

Wilkinson, D. G., Bailes, J. A. and McMahon, A. P. (1987). Expression of the proto-oncogene int-1 is restricted to specific neural cells in the developing mouse embryo. *Cell* **50**, 79–88.

Wilkinson, D. G., Peters, G., Dickson, C. and McMahon, A. P. (1988). Expression of the FGF-related proto-oncogene int-2 during gastrulation and neurulation in the mouse. *EMBO J.* **7**, 691–695.

**10**
**Retrov**
**proc d**

D. VALERI

*Gene Therapy*
*Immunology* -

Retroviru
Basic
Vect

Pack

Strat
Gene ther
Targ
Precl
(
(
I
/
I
Summary
Acknowlec
References

Our increasing
identify the ge
expected that t
and characteri

TRANSGENIC ANIM
ISBN 0-12-304530-4